Prenatal Diagnosis of Oculocutaneous Albinism by Analysis of the Fetal Tyrosinase Gene

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Tyrosinase-negative oculocutaneous albinism, the most severe subtype of a heterogeneous group of albinism, is an autosomal recessive trait caused by mutations in the tyrosinase gene. Prenatal diagnosis had been made previously only by evaluating fetal skin obtained by biopsy, an invasive procedure that cannot be performed earlier than 19 weeks of gestation. A pregnant mother of a 9-year-old Japanese boy with tyrosinase-negative oculocutaneous albinism wanted a prenatal diagnosis. Polymerase chain reaction amplification and allele-specific oligonucleotide hybridization revealed that the child is homozygous and the parents heterozygous for the pathologic mutation of the tyrosinase gene in exon 2 (single base insertion) but not for the one in exon 1. Prenatal diagnosis was made by analyzing the tyrosinase gene in fetal cells obtained by amniocentesis at 14 weeks of gestation, which demonstrated that the fetus was heterozygous for mutant tyrosinase gene. Pregnancy was therefore continued and a normal male infant was born.

This procedure, the analysis of the fetal genomic tyrosinase DNA, is a rapid and reliable approach to the prenatal diagnosis of oculocutaneous albinism at a relatively early stage of pregnancy and is safer and less invasive than previous methods using fetal skin biopsy. Key word: gene analysis. J Invest Dermatol 103:104–106, 1994

Oculocutaneous albinism (OCA), an autosomal recessive hereditary disorder of melanin metabolism, is characterized by a decrease in melanin in the skin, hair, and eyes [1,2]. Tyrosinase-negative OCA, the most severe subtype of a heterogeneous group, is caused by pathologic mutations in the tyrosinase gene [3]. Tyrosinase, a key enzyme for the biosynthesis of melanin in pigment cells, catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA). Since the first report of a mutation that produces tyrosinase-negative OCA [4], more than 25 alleles, each with a different mutation, have been found in patients with OCA [3]. Of these, only two major tyrosinase gene mutations have been found in Japanese patients [4,5], i.e., a single base insertion in exon 2 that shifts the reading frame and introduces a premature termination codon (TGA) following amino acid residue 298 (codon 316) [4] and a single base mutation in exon 1, causing an arginine to glutamine substitution at position 59 (condon 77) [5].

Tyrosinase-negative OCA leads to visual disturbances due to optic neurologic defects, photosensitivity, and the development of various malignant skin tumors [6,7]. The patient may be severely handicapped socially, especially in non-Caucasian countries.

The prenatal diagnosis of OCA had previously been possible only by analyzing fetal skin obtained at biopsy. Not only is biopsy of fetal skin more invasive than amniocentesis, but it cannot be performed earlier than 19 weeks [8,9]. Our objective was to establish a new strategy for the prenatal diagnosis of OCA at an earlier stage of pregnancy with a less invasive procedure. We present the successful prenatal exclusion of tyrosinase-negative OCA by analysis of the fetal tyrosinase gene at an early stage of pregnancy.

MATERIALS AND METHODS

Electron Microscopic 1-3,4-Dihydroxyphenylalanine (DOPA) Reaction Test The presence of tyrosinase activity was evaluated in a 9-year-old Japanese boy with typical clinical features of OCA. His mother was in her second pregnancy, and decided to proceed with the pregnancy only if a prenatal examination would confirm no evidence of OCA. The boy had white hair, light blue eyes, nystagmus, and poor visual acuity. The tyrosinase activity of his epidermal melanocytes was evaluated by the electron microscopic DOPA reaction test using the skin sample obtained by biopsy, as previously described [10]. In brief, the small skin samples were fixed with 2% glutaraldehyde and incubated in 0.1% DOPA/phosphate buffer for 4 h at 37°C. The sample was then postfixed in 1% osmium tetroxide for 1 h. After dehydration, the specimen was embedded in Epon. Ultrathin sections were cut and investigated in an electron microscopy. As a control, we used a sample of skin obtained from a tyrosinase-positive patient with OCA who was previously confirmed to show tyrosinase positivity on the electron microscopic DOPA reaction test.

Analysis of Tyrosinase Gene We initially determined whether the 9-year-old boy with OCA, his parents, and the members of his family possessed either of the two major pathologic mutations of the tyrosinase gene known in Japanese patients with tyrosinase-negative OCA. Blood samples were taken from the boy with OCA, as well as his parents, grandparents, and grandparents, three aunts, one uncle, and five cousins. DNA was extracted from the peripheral blood by the phenol method and amplified by the polymerase chain reaction (PCR). The conditions for PCR were basically the same as those described previously for exon 1 [5] and exon 2 [4]. In brief, 868-base pair (bp) and 118-bp sections of the respective tyrosinase exons 1 and 2 were amplified using specific primers and Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The synthetic primers SP1F (5'-CGCGAACCTTGTGGGACT-3') for the sense strand 54 to 73 and SP2R (5'-GTATATCTAGTACATATCC-3') for the antisense strand 902 to 921 were used for the partial exon 1 amplification. The synthetic primers SP3F (5'-ATTGTCTGTAGCCGATTGGAGG-3') for...
the sense strand 902 to 923 and SP4R (5'-CTTCCAGTGTTATTCCTAAAGCCT-3') for the antisense strand 1097 to 1118 were also used for partial exon 2 amplification. Denaturing, annealing, and polymerizing steps were performed at 94°C for 1 min, 50°C for 2 min, and 73°C for 3 min, respectively [11]. These steps were repeated for 30 cycles. Subsequently, 10 μl of the PCR products were dot-blotted onto the same four nylon membranes for the detection of normal or mutant alleles in both exons 1 and 2. The products on two membranes were hybridized with either 32P-labeled, exon 1-specific normal (ST1N: 5'-CCACGACTCCTGGTGCTATCA-3') or mutant (ST1M: 5'-CCACGACTCCTGGTGCTATCA-3') oligonucleotide probes for the antisense nucleotide sequence 302 to 321 in the presence of a fivefold amount of nonlabeled competitive sequences and with gradient temperatures from 60°C to 40°C, as described previously [12,13]. The other two membranes were also hybridized with either 32P-labeled exon 2-specific normal (ST2N: 5'-GAGGCTTGGGGTTCTGGAT-3') or mutant (ST2M: 5'-GAGGCTTGGGGTTCTGGAT-3') oligonucleotide probes for the antisense nucleotide sequence 1000 to 1019, as above.

**Prenatal Diagnosis**

Amniocentesis was performed in the 14th week of gestation to obtain fetal cells. Fetal cells were routinely cultured in Falcon flasks and maintained in Chang media (Hana Biologicals, Alameda, CA). At confluence on day 7, cells were dislodged mechanically from the flasks using a scraper. Genomic fetal DNA was extracted, amplified, and blotted onto the nylon membrane as described above. Allele-specific oligonucleotide hybridization was also performed as above.

**RESULTS**

**Electron Microscopic DOPA Reaction Test**

Electron microscopic DOPA reaction test of the skin sample of the 9-year-old boy with OCA demonstrated only melanosomes of stage I and II, even after incubation in the DOPA solution, and there was no further melanization of the premature melanosomes in melanocytes (Fig 1A). Stage II melanosomes were oval and measured about 300 nm long and 100 nm wide. They often presented a characteristic matrix consisting of parallel longitudinal and transverse filaments (Fig 1A). The premature melanosomes in the skin of two control Japanese patients with tyrosinase-positive OCA were clearly melanized to stage IV following incubation in DOPA solution (Fig 1B). These results confirmed the lack of tyrosinase activity in the melanocytes of this 9-year-old boy.

**Analysis of Tyrosinase Gene**

No nucleotide mutation was found in exon 1 in the DNA of the 9-year-old boy with tyrosinase-negative OCA or in any members of his family, including the parents and the 12 other relatives examined (Fig 2). Thus, the normal, but not the mutant, probe hybridized with the amplified DNA of all the family members. The mutant probe for exon 2 hybridized with the amplified DNA of the patient and his parents, grandmothers, three aunts, and one cousin, but not with that of his grandparents, one uncle, and two cousins. The normal probe for exon 2 hybridized with the amplified DNA of all samples except that from the proband (Fig 2).

These data confirm the autosomal recessive inheritance of OCA in the family pedigree. The data also indicate that the patient's tyrosinase gene indeed contained an insertion of a C residue in both alleles, resulting in a homozygous condition for the mutant tyrosinase gene. His parents, grandmothers, and several relatives, but not his grandparents, were confirmed to be heterozygous and thus were expected to be carriers of the OCA trait.

**Prenatal Diagnosis**

Based on data from the tyrosinase gene mutation of the patient and his family members, prenatal diagnosis was performed using DNA extracted from the fetal cells obtained by amniocentesis. No nucleotide mutation was found on exon 1 in the fetal DNA (number 16 of Fig 2). Amplified fetal DNA hybridized with both mutant and normal probes for exon 2 of the tyrosinase gene, confirming that the fetus was not homozygously affected but was a heterozygous carrier of OCA. Pregnancy was therefore continued and a normal male infant was delivered. He had black hair and eyes and no evidence of OCA.

**DISCUSSION**

Our results confirm that the analysis of the tyrosinase gene by PCR amplification and allele-specific oligonucleotide hybridization enables prenatal diagnosis of tyrosinase-negative OCA. This can be done at a relatively early stage of pregnancy, as early as 8–10 weeks of gestation by chorionic villus sampling, or at 12–14 weeks of gestation by amniocentesis. In the present case, when we could confirm the mutation in exon 2 of the tyrosinase gene in this family, the mother was already in her 14th week of pregnancy. We therefore obtained fetal cells by amniocentesis, the safest option at this stage of pregnancy. This technique also provided a quick and reliable diagnosis of the patients and carriers with simple blood sampling.

The prevalence of OCA in Japan is about 1 in 20,000 people. Once the non-Caucasian parents have an albino child, they usually do not wish to continue the pregnancy with an affected fetus. In fact, the parents of this 9-year-old boy with OCA, like other parents in our experience [14], had decided to discontinue the pregnancy if a prenatal diagnosis could not be obtained.

The first prenatal diagnosis of OCA was made in 1983, in which a lack of stage IV melanosomes in hair bulb melanocytes of the fetal scalp obtained at 20 weeks gestation was confirmed by electron microscopic observation [15]. The technical difficulty of this method is that the skin must be taken from the scalp of the fetus; hair bulb melanocytes must be located by serially sectioning small samples of skin [16]. To overcome this technical problem, we recently introduced an electron microscopic DOPA reaction test that...
enables easier and more accurate prenatal diagnosis using a specimen of fetal skin from any body area [14]. However, such methods [14–16] require fetal skin biopsy, which is invasive and cannot be performed earlier than 19 weeks of gestation.

Tests for the tyrosinase gene in the boy with OCA and his family members confirmed the availability of analysis of the fetal tyrosinase gene for prenatal diagnosis. We indeed excluded OCA using amniotic cells, not with fetal skin, at 14 weeks of gestation. In the analysis of the tyrosinase gene, hybridization was performed under conditions in which the temperature was decreased gradually from 60°C to 40°C. In this way we could omit the cumbersome procedure of determining the proper hybridization temperature for each probe and each membrane [12,13]. Furthermore, by adding an excess amount of fivefold competitive sequences in addition to labeled oligonucleotides, nonspecific binding could be markedly reduced to obtain clear results. Restriction fragment-length polymorphism can be a future alternative method for prenatal diagnosis, although further study will be necessary.

In conclusion, analysis of the fetal tyrosinase gene by PCR amplification and allele-specific oligonucleotide hybridization enables the prenatal diagnosis of tyrosinase-negative OCA at an earlier stage of gestation. Because only amniocentesis is required, this technique is safe and less invasive and can provide a rapid and reliable prenatal diagnosis of OCA at an early stage of pregnancy.

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